DLS ECHO Biosafety Session: June 27, 2023

Laboratory Acquired Infections

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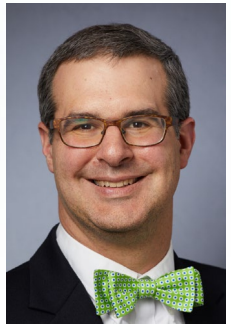
Professor of Laboratory Medicine, Yale School of Medicine
Associate Chief for Laboratory Medicine, VA Connecticut Health Care
New Haven, CT



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New Haven, CT

CDC Centers for Disease Control and Prevention
UNM Health Sciences
Project ECHO

Agenda

- Didactic and Case Presentation
- Discussion
- Summary of Discussion
- Closing Comments and Reminders

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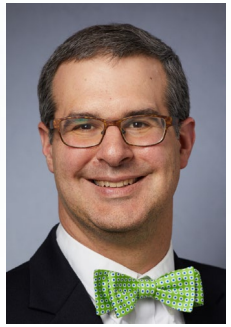

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DLS ECHO Biosafety Session: July 18, 2023

Safety Implications of Diagnostics in Remote Areas

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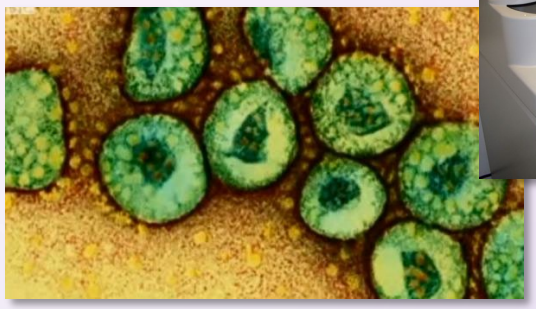

LABORATORY-ACQUIRED INFECTIONS AND MEDICAL LABORATORY BIOSAFETY

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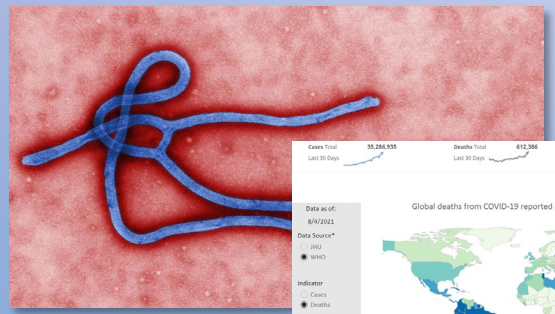

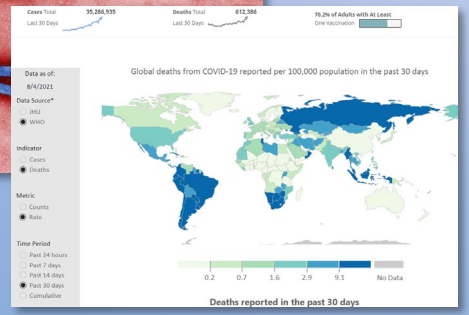

OBJECTIVES

PARTICIPANTS WILL BE ABLE TO (ACTUALLY, THAT'S JUST A WISH, I'M NOT SURE I'M REALLY ABLE TO, BUT LET'S PRETEND, SHALL WE?)

- DISCUSS POTENTIAL ROUTES OF ACQUISITION OF INFECTIONS IN THE CLINICAL LABORATORY
- RECOGNIZE HISTORICAL EXAMPLES OF CLINICAL LABORATORY ACQUIRED INFECTIONS
- DESCRIBE THE MAJOR CURRENT GAPS IN BIOSAFETY KNOWLEDGE AND PRACTICE IDENTIFIED AFTER EBOLA AND COVID
- IN THAT CONTEXT, DISCUSS A COUPLE OF CASES.

'The Seventh Plague of Egypt', by John Martin (England, 1823)
From the collection of the Museum of Fine Arts, Boston

Major source:
Cornish NE, Anderson NL, Arambula DG, Arduino MJ, Bryan A, Burton NC, Chen B, Dickson BA, Giri JG, Griffith NK, Pentella MA, Salerno RM, Sandhu P, Snyder JW, Tormey CA, Wagar EA, Weirich EG, Campbell S. Clinical Laboratory Biosafety Gaps: Lessons Learned from Past Outbreaks Reveal a Path to a Safer Future. Clin Microbiol Rev. 2021 Jun 16;34(3)

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INFECTION IN CLINICAL LABORATORIES

SOME CASES, MANY UNKNOWNS

RISKS IN PERSPECTIVE: AIDS EPIDEMIC, 1980S

- **LESS THAN 3% OF HEALTHCARE WORKERS** ARE LABORATORY PROFESSIONALS
- INTERACT WITH POTENTIALLY INFECTIOUS MATERIAL FROM **MANY MORE PATIENTS** THAN OTHER HEALTHCARE WORKERS
- **ALMOST 30% OF OCCUPATIONALLY ACQUIRED INFECTIONS OF HIV**, 1981-2010, AFFECTED CLINICAL LABORATORY WORKERS
- ON A PER CAPITA BASIS, THE RISK OF AN ACCIDENTALLY ACQUIRED INFECTION TO A CLINICAL LABORATORY WORKER MAY BE **HIGHER BY A FACTOR OF 10** THAN FOR A NON-LABORATORY HEALTHCARE WORKER
- ON THE OTHER HAND, **NO SARS** IN CLINICAL LABORATORY WORKERS. LAB SAFETY PROGRESS OR LUCK?

Healthcare Personnel with Documented and Possible Occupationally Acquired HIV Infection, by Occupation, 1981-2010

Occupation	Documented	Possible
Nurse	24	36
Laboratory worker, clinical	**16**	**17**
Physician, nonsurgical	6	13
Laboratory tech, nonclinical	3	-
Housekeeper/maint worker	2	14
Technician, surgical	2	2
Embalmer/morgue technician	1	2
Health aide/attendant	1	15
Respiratory therapist	1	2
Technician, dialysis	1	3
Dental worker, incl dentist	-	6
Emerg med tech/paramedic	-	12
Physician, surgical	-	6
Other tech/therapist	-	9
Other healthcare occ	-	6
Total	**57**	**143**

Table 1. Some microbiologists or people who worked in fields related to microbiology who contracted the disease in which they worked (see text for details of each case)

Disease	Causal agent	Researcher or worker infected	Year
Brucellosis	*Brucella melitensis*	Florence Nightingale[s]	1855
	Brucella melitensis	Jeffrey A. Marston[s]	1861
	Brucella melitensis	Alice C. Evans[s]	1922
Carrion's disease	*Bartonella bacilliformis*	Daniel Alcides Carrión[d]	1885
	Bartonella bacilliformis	Ovidio García Rosell[s]	1928
	Bartonella bacilliformis	Maxime Kuczynski-Godard[s]	1937
Cholera	*Vibrio cholera*	Louis Thuillier[d]	1883
	Vibrio cholera	Max von Pettenkofer[s]	1892
Epidemic typhus	*Rickettsia prowazekii*	Howard Taylor Ricketts[d]	1910
	Rickettsia prowazekii	Stanislaus von Prowazek[d]	1915
Rocky Mountain spotted fever	*Rickettsia ricketsii*	Thomas Bailey McClintic[d]	1912
	Rickettsia ricketsii	Henry Cowan[d,f]	1924
Yellow fever	Yellow fever virus	Elihu H. Smith[d]	1798
	Yellow fever virus	Jesse W. Lazear[d]	1900
	Yellow fever virus	James Carroll[s]	1900
	Yellow fever virus	Adrian Stokes[d]	1927
	Yellow fever virus	Hideyo Noguchi[d]	1928
	Yellow fever virus	William A. Young[d]	1928

BEING FAMOUS OR PROMINENT IS NO PROTECTION

- Ricketts and Prowazek both died of their namesakes.
- Both killed by epidemic typhus; Ricketts while studying the disease in Mexico, Prowazek in Germany.
- Many others, of course
- Piqueras M. Microbiology: a dangerous profession? Int Microbiol. 2007 Sep;10(3):217-26.

HOW MANY CLINICAL LABORATORY-ACQUIRTED INFECTIONS ARE THERE?

We have no idea!!

There is no reporting system.

There are disincentives to report laboratory-acquired infections (LAIs)

LABORATORY ACQUIRED INFECTIONS IN CLINICAL LABORATORIES

- ClinMicroNet online survey of clinical laboratory directors, 2002-04
  - 53 large hospital labs, 32 smaller hospital labs, 3 national diagnostic reference labs
- 33% of clinical laboratories reported at least one laboratory acquired infection (LAI)
  - 41 Bacterial LAIS reported
- E.J. Baron and J.M. Miller, 2008, "Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks," *Diagn Microbiol Infect Dis*, 60 (3), 241-6

- Shigella (15)
- Brucella (7)
- Salmonella spp. (6)
- Staphylococcus aureus (6)
- *N. meningitidis* (4)
- *E. coli 0157:H7* (2)
- *C. difficile* (1)

LABORATORY ACQUIRED INFECTIONS CONTINUE

- **Laboratory-acquired meningococcal disease** -- United States, 2000
- **Laboratory-acquired West Nile virus infections** -- United States, 2002
- **Laboratory-acquired brucellosis** -- Indiana and Minnesota, 2006
- **Laboratory-acquired vaccinia virus infection** -- Virginia, 2008
- **Fatal laboratory-acquired infection with an attenuated *Yersinia pestis* strain** -- Chicago, Illinois, 2009

- These are predominantly research laboratories though.

WHERE MIGHT CLINICAL LABORATORY ACQUIRED INFECTIONS COME FROM?

ROUTES OF EXPOSURE

Table 1. Common laboratory routes of exposure to infectious agents

Route	Microbiological practices/accident
Inhalation	Procedures that produce aerosols:
	Centrifugation
	Mixing, sonication, vortexing, blending
	Spills and splashes
	Pouring/decanting culture fluids
	Manipulation of inoculating loop
Inoculation	Needlestick
	Lacerations from sharp objects (e.g., blades, broken glass)
Ingestion	Splashes to the mouth
	Placing contaminated articles/fingers in mouth
	Consumption of food in the laboratory
	Mouth pipetting
Contamination of skin and mucous membranes	Splashes Contact with contaminated fomites

- FROM: SEWELL DL. (2006) LABORATORY-ACQUIRED INFECTIONS: ARE MICROBIOLOGISTS AT RISK? CLINICAL MICROBIOLOGY NEWSLETTER 28:1

CLINCAL LABORATORY RISK: INSTRUMENTATION AND PROCESSES

PRE-ANALYTIC

SAMPLE COLLECTION

TRANSPORT

RECEPTION AND UNPACKING

CENTRIFUGATION

UNCAPPING

ALIQUOTING

TRANSPORT WITHIN THE LAB

TRANSPORT TO REFERENCE LABS

ANALYTIC

CHEMISTRIES

BLOOD GASES

HEMATOLOGY

BACTERIOLOGY

VIROLOGY

MOLECULAR TESTING

TRANSFUSION MEDICINE

POST-ANALYTIC

WASTE MANAGEMENT

SAMPLE STORAGE - RETRIEVAL

RISKS IN THE ANALYTIC PHASE 1

- Chemistry
  - Complex analyzers with multiple sampling stations, aliquoting events, and waste pathways.
    - Many cannot perform closed-tube sampling
    - Require frequent periodic maintenance, service.
    - Extremely expensive; critical for care of large numbers of patients.
- Blood Gases
  - Sample submitted in syringe
  - Extremely labile sample requires rapid handling
- Hematology
  - Complex analyzers as above
  - Manual or automated slide-making; glass slides.

RISKS IN THE ANALYTIC PHASE 2

- Bacteriology
  - Survival of emerging viruses in culture media generally unknown, but likely (old studies show HIV does)
  - Much manual handling of samples and cultures
  - Complex analyzers as above
- Virology
  - Growth of emerging pathogens in viral culture (waning in importance as labs abandon viral culture)
- Molecular diagnostics
  - Complex analyzers as above.
  - Many manual or semi-manual methods in some laboratories.
  - How to validate EUA tests for dangerous, rare pathogens?

RISKS IN THE ANALYTIC PHASE 3

- Transfusion Medicine
  - Tube-based methods likely generate droplets
  - No sealed-rotor blood bank centrifuge is currently available, per my local colleague.
  - Risks associated with gel or instrumented methods unknown.

BIOSAFETY ISSUES RAISED BY EBOLA (AND EMPHASIZED BY COVID…)

- Issues and risks fall into four groups
  - Biosafety gaps common across clinical laboratories
  - Gaps unique to specific areas
  - Systemic gaps in biorisk management as applied to clinical laboratories
  - Specific lessons-learned from Ebola in 2014-15.

BIOSAFETY GAPS COMMON ACROSS CLINICAL LABORATORIES

1. Laboratories lack direct control over how specimens are collected and transported
2. Our knowledge of instrument contamination during routine use, or during use with highly pathogenic microbes, is limited, and these risks may be under appreciated.
3. There is a lack of knowledge of and planning for decontamination of laboratory instruments.
4. There are discrepancies between the current designation of Category A infectious substances and the actual wide range of waste materials generated during clinical laboratory testing. These impact waste management before and after waste leaves the laboratory.
5. There is inadequate guidance or training for clinical laboratory professionals in use of PPE. The availability of PPE in clinical laboratories is often insufficient. There remains confusion between the differences in using PPE for direct patient care and the processes for PPE use in a clinical laboratory and testing environment.
6. it is often challenging for providers of laboratory safety training to collect evaluation data beyond learner satisfaction.
7. Data are lacking on to what extent laboratories conduct monitoring and evaluation of biosafety practice.

GAPS UNIQUE TO SPECIFIC AREAS

1. Blood Banks have unique biosafety concerns and provide critical supportive care.
2. Core Laboratories contain risks distinctly different from other areas, particularly risks related to automation.
3. Microbiology laboratories issues include blood culture instrument platforms, difficulty in switching from an automated to a manual testing method, and the viability of pathogens during and after preparation of malaria smears and Gram stains.
4. Anatomic Pathology may involve regular manual contact with large volume/mass specimens with high titers of unknown and/or known pathogens.

SYSTEMIC GAPS IN BIORISK MANAGEMENT AS APPLIED TO CLINICAL LABORATORIES

1. Clinical laboratories often don't know about the presence of infectious agents in specimens. Most research-oriented guidance is built around known hazards.
2. Current laboratory safety interventions often focus on rare events and preventive efforts, whereas systems for monitoring and evaluating safety interventions analogous to those for quality improvement are usually absent.
3. The absence of a full biorisk management cycle (assessment, mitigation, and performance evaluation) in clinical laboratories.
4. There is no surveillance system for laboratory acquired infections, and there are disincentives to reporting.
5. There is a lack of evidence-based research and publications focused on biosafety.
6. The regulatory framework for clinical laboratory safety is weak and limited, and nonexistent for waived labs.
7. Biosafety guidelines inadequately address risks in specialty areas of the laboratory.

SPECIFIC LESSONS-LEARNED FROM EBOLA IN 2014-15

1. Gaps were identified in all phases of testing; pre-analytical, analytical, and post-analytical.
2. Laboratories needed help developing testing menus for PUI for EVD that emphasized the need to maximize diagnostic yield
3. ethical challenges included how to balance the duty to provide laboratory services for routine patient care and suspected patients, and laboratory personnel protection. Ethical codes and frameworks for laboratory practice are lacking.
4. Inconsistencies in recommendations from different sources further contributed to confusion among laboratory professionals about the degree and nature of the risks.

LESSONS FROM COVID-19

- Data is Still of First Importance
  - Early paper suggested there was little viruria or viremia. And thank goodness for that.
  - I have no idea how we would have handled COVID had there been significant virus in common specimen types, especially early in the pandemic.
- Other Workers are Part of the Risk
  - With a disease as widespread as COVID-19, person-to-person transmission in the work environment may be the dominant risk.

SO, RISKS EXIST. WHAT NOW?

"They're for emotional protection."

NEEDS

- Research on:
  - Hazards associated with modern clinical laboratory equipment; but who'd fund it?
  - Surveillance of laboratory-associated infections.
  - Clinical laboratory safety improvement.
  - Impact of delays in testing on care for patients at-risk of emerging infections, and balancing risks to patients and staff.
- Standards related to:
  - Clinical laboratory risk assessment.
  - Emerging infection preparedness and safety.
  - Instrument safety and decontamination.

THE PATH FORWARD

- Enhanced national oversight for clinical biosafety.
- Improvements in instrument design.
- More training materials and resources
- Guidance in laboratory workflow in cases of emerging infections; pre-analytical, analytical, and post-analytical.
- Creation of a surveillance system for laboratory-acquired infections.
- Biosafety research in PPE, engineering controls, facility design, workflow and process design, pathogen inactivation in typical matrices, POC versus laboratory-based testing.
- Guidance on waste management and specimen storage.

Microbiology laboratory exposures to *Brucella melitensis:* YNHH experience with two cases

David R. Peaper, MD, PhD, D(ABMM)
Associate Professor of Laboratory Medicine
Director, Clinical Microbiology Laboratory, Yale New Haven Hospital

Goals and Objectives

- Present two cases of clinical microbiology laboratory exposures to Brucella.
- Discuss factors identified that were associated with laboratory exposures.
- Identify areas where clarity could be provided in laboratory guidance about identifying potential cultures with select agents.
- (Briefly) discuss the select agent program.

Case Presentation 11/18 to 12/5

- 8 year old with multiple visits to PCP and off-site ED (11/18) for fevers, vomiting, abdominal pain, loss of appetite
  - Slight lab abnormalities, minimal infectious work-up
  - Clinical Dx = Viral Syndrome
- Presents to YNHH ED 11/21
  - Still having fevers, abd pain, near daily vomiting
  - Now reporting thigh pain (“shooting”), dorsal foot pain when walking, headache
  - Mother reports that the family traveled to Egypt in August (previously denied travel)

Review of Systems

- General: Anorexia (no wt loss), change in activity, fatigue, fever/chills, diaphoresis
- HEENT: sore throat (last week); No congestion, rhinorrhea, or sneezing
- Resp: No cough or dyspnea
- GI: abdominal pain, nausea and vomiting; No occult blood or diarrhea
- GU: No for dysuria or hematuria
- Musculoskeletal: myalgias
- Neurological: headaches

Physical Exam

- Vitals: **Temp** 97 (36.1 C); **Pulse** 107; **BP** 88/54; **Resp** 20; **O2** 97%
- General: well-developed and well-nourished
- HEENT: MMM, Oropharynx clear, PERRLA, EOM WNL, Conjunctivae normal
- Neck: Normal ROM
- Cardiovascular: RRR, S1 normal. Palpable pulses
- Respiratory: Intermittent wheezes, Effort normal
- Abdominal: Bowel sounds WNL, hepatosplenomegaly, RUQ tenderness
- Neuro: AAOx3
- Skin: Warm/dry, Capillary refill takes less than 3 seconds, No jaundice

Follow Up

- Multiple admissions and discharges between 11/21 and 12/5
- Blood cultures collected 11/21 and 11/22
- Progressively developed liver lesions suggestive of abscesses
- Abdominal MRI show lesions suggestive of osteomyelitis
- GI symptoms continued

Differential Diagnosis for ID and Travel to Egypt

- Now 10 weeks out from travel
  - Malaria: Fever, chills, sweats, HA, N/V, body aches, malaise (incubation: 7-30 days)
  - Typhoid: fever, fatigue, rash, HA, malaise, abd pain, anorexia, hepatosplenomegaly, Diarrhea, Constipation (Incubation: 6-30 days)
  - Yellow Fever : Most Asymptomatic; Fever, chills, HA, back ache, N/V, myalgia; jaundice and hemorrhagic symptoms in 15%(incubation: 3-6 days)
  - Hepatitis A: Abd pain, fever, malaise, anorexia, nausea, jaundice (incubation: Avg 28 days)
  - Zika: Asymptomatic; Fever, Rash, joint/muscle pain, conjunctivitis, HA (Incubation: few days)
  - Dengue : Fever, HA, Nausea/vomiting, Rash, Eye/joint/muscle pain (incubation: 2 wks)
  - Schistosomiasis: Rash, fever, HA, myalgia, respiratory symptoms, Hepatosplenomegaly, dysuria, hematuria (incubation: 14-84 days)

Laboratory Evaluation

- Blood Cultures:
  - Order 1: 2/2 bottles with *Bacillus* spp. (not *B. anthracis*) and CoNS
  - Order 2: 1/2 bottles with GNR → *Ochrobactrum anthropi* (99.9% match via MALDI-TOF)
- Stool PCR → *Yersinia enterocolitica*
- Other extensive ID work-up unrevealing:
  - Malaria smear, HAV serology, Schistosoma serology, CMV, EBV Ab
- Non-ID workup unrevealing
- Brucella serologies ordered on day of discharge

Clinical picture: Brucella?

Pros

- Clinical Syndrome - Hepatosplenomegaly, Fever, Bone lesion

Cons

- No reported high risk exposures
- No symptoms until 10 weeks after return from Egypt
- Cultures Grew in 3 Days
- Adequate growth in culture by 24 hours for ID
- MALDI-TOF ID 99.9%

Brucella Ab Studies: Reactive at 1:1280

Immediate Follow Up

- Lab alerted to concern for Brucellosis → Pending cultures flagged for special handling
- Repeat blood cultures 12/5 grew small GNR → Could not rule-out Brucella → Sent to CT State Lab → PCR Positive for *Brucella* spp.
- Also sent isolate from 11/21 → PCR Positive for *Brucella* spp.
- Species confirmed as *Brucella melitensis* at CDC
- Exposure investigation undertaken

Reaction to CDC Testing in Laboratory

Potential Audience Response Questions

- What criteria should the clinical lab consider when assessing the risk of a culture containing Brucella?
  - GNR + No Growth on MAC
  - GNR + No Growth on MAC + Growth Characteristics on Blood and Choc
  - Gram stain reaction (faint staining GN coccobacilli)
  - You know it when you see it

APHL Select Agent Guidance - Brucella

Gram Stain

- Faintly staining, not clustered, tiny Gram negative coccobacilli (0.4 µm-0.8 µm)
- May retain crystal violet stain and may be mistaken for Gram positive cocci

Biochemical/Test Reactions

- Catalase, oxidase and urea positive

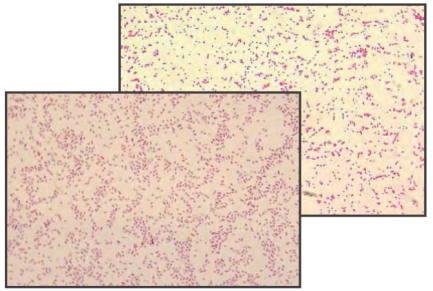
  Note: Oxidase may be variable and test should be performed on fresh cultures (18-24h)
- *S. aureus* streak negative (X & V Factor satellite test)

Colony Morphology

- Aerobic, slow growth
- Slow growth seen on BAP and CHOC (CO_2 may be required by some strains)
- Poor to variable growth on MAC. Pinpoint colonies may infrequently be observed with some strains after extended blood culture incubation (7 days)
- Non-mucoid
- Pinpoint colonies at 24h, and easily visible, discrete, white, non-hemolytic colonies at 48h (0.5 mm-1 mm)
- Colonies on BAP have no distinguishing features. They will appear as white, non-pigmented and non-hemolytic. Colonies will appear as raised and convex with an entire edge and shiny surface

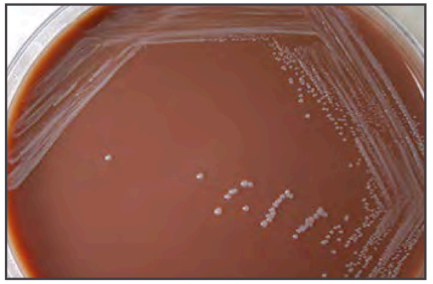
Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., "slow growing" *Staphylococcus* spp., *Oligella ureolytica, Bordetella bronchiseptica, Haemophilus* spp., *Pasteurella* spp., *Psychrobacter phenylpyruvicus* and *Psychrobacter immobilis.*



Gram Stain

48h growth on BAP



72h growth on CHOC

APHL Brucella Algorithm

Gram stain morphology

☐ Faint staining, not clustered, tiny (0.4 x 0.8µm), Gram negative coccobacilli?
Note: May retain crystal violet stain and be mistaken for Gram positive cocci

Growth

☐ Subculture positive aerobic blood culture to BAP, CHOC?

☐ Aerobic, slow, poorly growing colonies after 24h incubation in 5-10% CO_2 at 35°C?
Note: Incubate plates for at least two additional days if no growth in 24h.

☐ Organism not growing on MAC?

☐ Slow growing in automated blood culture systems?
Note: Consider extended incubations up to 2-3 weeks.

YES TO ALL

Satellite negative at 24-48 hours?
Note: Spot BAP with *S. aureus* ATCC 25923

NO → *Brucella* spp. ruled out → Consider *Haemophilus*

YES

Oxidase and catalase positive?

NO → *Brucella* spp. ruled out → Consider *Francisella*
Refer to ASM sentinel procedures

YES

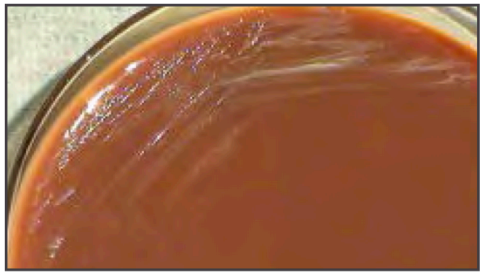
Urea positive?

NO → *Brucella* spp. ruled out → Reincubate
Use internal laboratory procedure

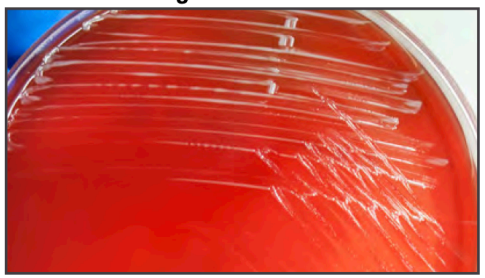
YES, STOP

***Brucella* spp. not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Brucella* spp. submitted to LRN Reference Level Laboratory for confirmatory testing.

NO TO ANY



24h growth on CHOC



48h growth on BAP

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ASM Brucella Guidelines

Major Characteristics of *Brucella* species

Gram Stain Morphology: Small (0.4 x 0.8 µm), Gram-negative coccobacillus

THINK BRUCELLA

Growth: Subculture positive aerobic blood culture bottle to:
BAP, CHOC
Incubate in 5-10% CO_2 at 35°C

Spot BAP with *S. aureus* ATCC 25923 for satellite test.

Note poorly growing colonies after 24 hours incubation on BAP and CHOC.
Incubate plates for at least 2 additional days if no growth in 24 hours.
Organism does **not** grow on MAC.

Review of Microbiological Findings from 11/21 and 11/22

- Blood cultures collected 11/21 and 11/22
  - Order 1: Aerobic and Anaerobic bottles with *Bacillus* spp. (not *B. anthracis*) and CoNS after 18 to 24 hours
  - Order 2: Aerobic bottle with GNR (growth w/in 60 hours)
    - Adequate / unremarkable plate growth on blood and choc next day → *Ochrobactrum anthropi* (99.9% match via MALDI-TOF)
    - Facultative anaerobic growth
    - No growth on MAC
    - Not handled under BSL-3 conditions; reviewed with clinical team at bench rounds

Audience Response Question

- In an open concept laboratory, how do you assess exposure risk for post-exposure prophylaxis and follow-up testing?
  - No idea. Consult with CDC / Inf Prev / Occ Health
  - Whole lab is considered exposed if no barriers
  - Worked within five feet of open culture plate

Occupational Exposure Case

Clinical isolate from YNHH lab on 11/21 specimen

- Employees from Yale New Haven Hospital and University were present in laboratory where sample was not handled under BSL3 conditions due to Brucella not being identified or suspected

CDC recommendations for lab exposure follow up:

- All person in lab where Brucella work occurred who handled the specimen or were within 5 feet of specimen were high risk and should have prophylaxis for 3 wks. (Doxycycline and Rifampin)
- Anyone present in the room greater than 5 ft. away from the work were low risk → discuss prophylaxis, especially consider prophylaxis if pregnant or immunocompromised

Additional follow up for exposed

- Sequential serum testing at 0, 6, 12, 18, 24 weeks
- Daily self fever check for 24 wks.
- Weekly symptom watch (required phone contact from health care provider) for 24 weeks
- Total number of exposed patients between the two institutions was 21

Outcomes

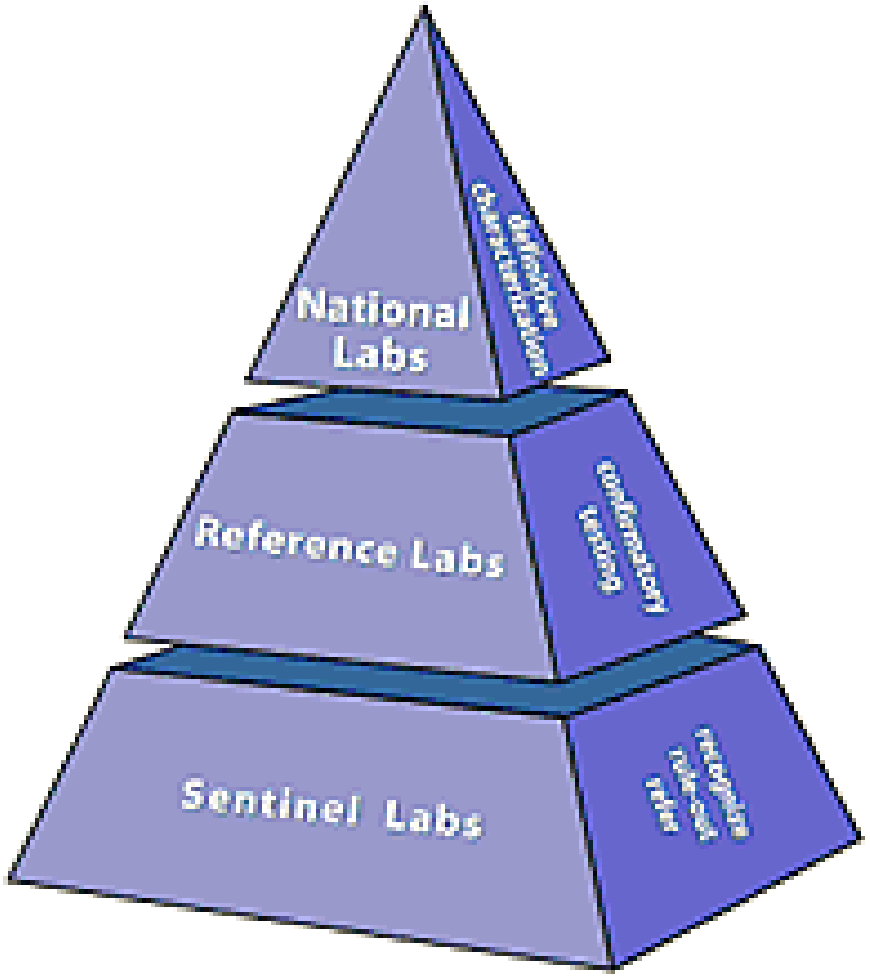
- No secondary cases of Brucella
- All initial and follow up titers for Brucella remained negative (a few titers read as indeterminate were considered negative by CDC zoonoses experts)
- No adverse outcomes in exposed patients who were pregnant or who sustained adverse reactions to the prophylactic antibiotics
- Hospital laboratory reviewed lab practices to help prevent future exposures

Select Agents

- Include toxins, viruses, bacteria, and fungi that "***have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plan products.***"
- Substantial overlap with "potential agents of bioterror"
- ***Most*** of the agents affecting humans will not be identified in the laboratory through any routine testing
- ***Most*** require specific testing → If suspected must coordinate with public health officials and laboratories

Laboratory Response Network (LRN)

- **National labs:**
  - Unique resources to handle highly infectious agents and the ability to identify specific agent strains.
- **Reference labs:**
  - Perform tests to detect and confirm the presence of a threat agent.
  - Ensure a timely local response
- **Sentinel labs:**
  - Hospital-based labs that have direct contact with patients.
  - First facility to spot a suspicious specimen.
  - ***Rule-out or refer a suspicious sample to the right reference lab.***



Bacterial Select Agents

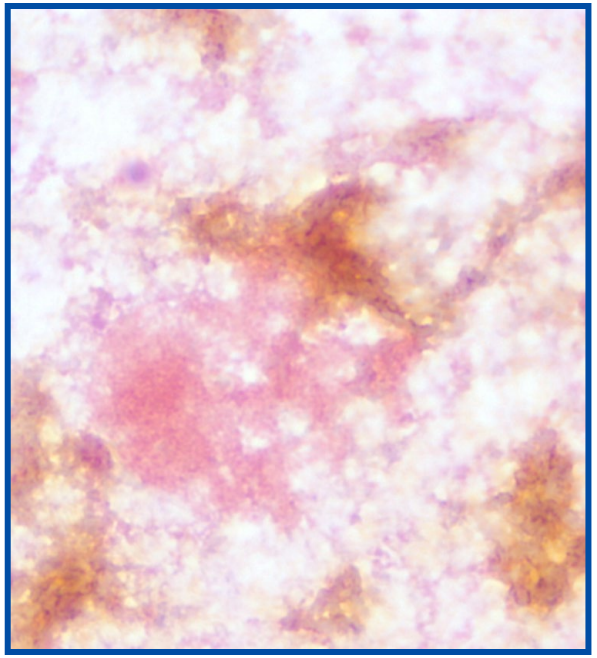
- All bacterial select agents will grow in the clinical microbiology using routine culture conditions.
- Recovery of select agents poses a risk to laboratory workers

Select Agent	Clinical Disease
Bacillus anthracis *Bacillus cereus* Biovar *anthracis*	Anthrax: cutaneous, inhalational, GI
Francisella tularensis	Tularemia
Yersinia pestis	Plague
Brucella spp.	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Meliodosis

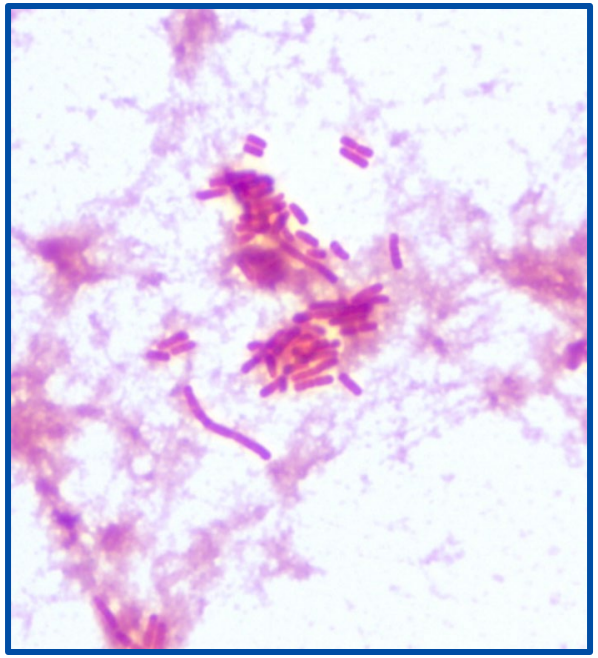
Growth Characteristics of Bacterial Select Agents

Organism	Gram Stain	Media Growth	Biosafety Precautions
Bacillus anthracis	Large Gram-positive rods	Blood + Chocolate + MacConkey -	BSL-2 (+BSC)
Brucella species	Faintly staining, tiny Gram-negative coccobacilli	Blood + (Slow) Chocolate + (Slow) **MacConkey -**	BSL-3 or BSL-2 with BSC and BSL-3 PPE
Burkholderia mallei	Faintly staining, Gram-negative bacilli or coccobacilli	Blood + Chocolate + **MacConkey +/-**	BSL-3 or BSL-2 with BSC and BSL-3 PPE
Francisella tularensis	Poorly staining, tiny Gram-negative coccobacilli	Blood +/- (Slow) Chocolate + (Slow) **MacConkey -**	BSL-3 or BSL-2 with BSC and BSL-3 PPE
Yersinia pestis	Gram-negative rods	Blood + Chocolate + MacConkey +	BSL-2 (+BSC)
Burkholderia pseudomallei	Gram-negative rods	Blood + Chocolate + MacConkey +	BSL-3 or BSL-2 with BSC and BSL-3 PPE

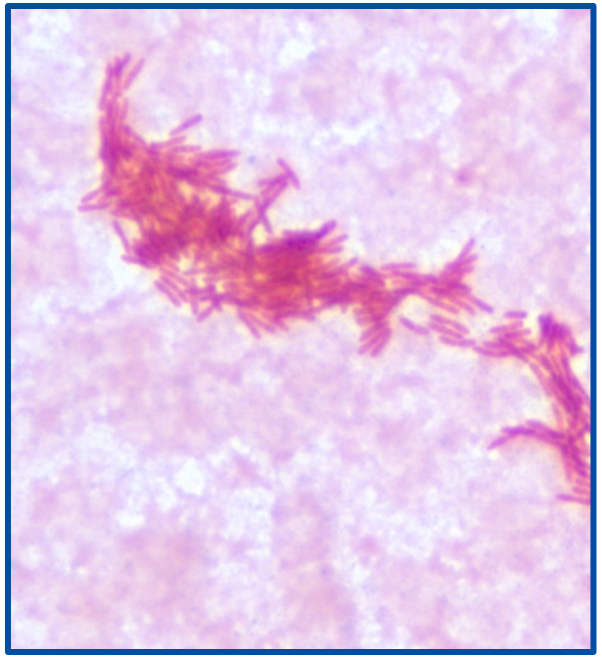
Gram Stain of Blood Culture Bottle



Brucella spp.



E. coli



P. aeruginosa

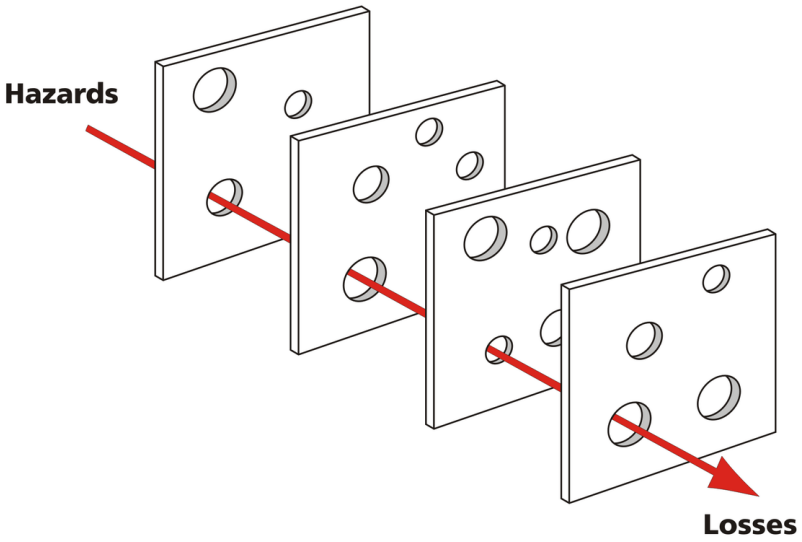
1000x magnification for all slides

Case Resolution

- After diagnosis, family told clinicians that patient had possible exposures
  - Spent time on a farm playing with goats and camels
  - Had unpasteurized dairy products
- Patient received 6 weeks of antibiotics
  - Planned for longer, but had severe side effects
  - Now being monitored for signs of recurrence

The Swiss Cheese Effect

- Family repeatedly and adamantly denied patient could have been exposed
- Brucella incubation period is 2-4 weeks; patient didn't display symptoms for 10 weeks
- Cultures grew quickly (less than 3 days)
  - Enough to go on MALDI
- MALDI identified Ochrobactrum
  - Select Agents are NOT currently in the MALDI Databases
  - Unlikely to be diagnosed in usual clinical practice, so not usually an issue
  - In this case, MALDI made the closest match it could- Ochrobactrum



Human factors that contributed

- In-lab protocol lacked specificity when to suspect Brucella
- "It's almost certainly not" =/= "It's definitely not"
  - Rare things are rare, but not impossible
- Circular logic
  - It can't be Brucella b/c we should have ruled out Brucella
- Incomplete communication across shifts, days, and staff (long weekend)
- *Ochrobactrum anthropi* is uncommon ID, and lab staff were not familiar with growth characteristics of *O. anthropi*
- Over-reliance on MALDI-TOF high %-age identifications

Brucellosis in the USA

- At least 20 years since our last known Brucella case
- Typically ~100 cases per year in USA
- 6 states account for 60% to 70% of cases per year (California, Texas, Arizona, Florida, Georgia, Illinois)
- Most states won't see a case in a typical year → even less common for a given institution or provider to see a case

Audience response question (select all that apply)

- What would you do in your lab to avoid cases like this in the future?
  - Re-educate staff on characteristics of select agents
  - Modify protocols to explicitly list when an isolate should be “ruled-out” (e.g. all GNR/GNC with no growth on MAC)
  - Implement sticker system to communicate unusual gram stain findings across staff
  - Other

Bonus Case

- Almost exactly 1 year later.
- 48 yo male recently traveled to Egypt, to visit friends and family (he is from Egypt but immigrated to the US in 2012).
- He was there from 10/1-10/13. He felt well while he was there, had no known sick contacts, did not eat undercooked meat or other food that was concerning to him.
- He stayed in the city (Cairo) and had no animal exposures.
- He felt well when he returned home and was in his USOH until 11/1 or 11/2 when he developed headaches and fatigue and malaise.
- Multiple health care encounters over next week w/ persistent fevers and night sweats w/o diagnosis or resolution. Presented to ED.

Bonus Case

- Blood cultures collected 11/12 @ 534 pm
- Blood culture flags “Positive” on 11/15 @1218 AM → “No Organisms Seen” on Gram Stain
  - Blind subculture to Blood, Choc, ANA Blood
- Plates with adequate growth on 11/16 → Reviewed by blood bench
- Review of gram stain morphology from plate → Small, faintly staining GN coccobacilli → Stop the line → Send to state
- Brucella confirmed by CT DPH on 11/17

Follow Up History

- After diagnosis, exposure risks in Egypt were revisited
  - Continued to deny contact with sheep, cows or drinking unpasteurized milk or cheese. Ate cooked lamb
  - His friend then spoke with him in Arabic and he obtained history of eating unpasteurized Parmesan cheese

Case 2 – What went wrong?

- Blind subculture procedure did not include MAC

- Responses:
  - 1) Add MAC to all blind subcultures from blood cultures
  - 2) Rearrange lab:
    - Move blood bench to far corner of lab away from all other routine work
    - Place BSC next to blood bench to remove any barriers to working up plates in BSC

Challenges to the clinical microbiology laboratory

- Select agents are rare, and are not often in the clinical or microbiological differential diagnosis
- Potential select agents should not enter the regular laboratory workflow ("Rule Out or Refer"), but rare organisms are often not considered before an unusual ID is made
- All IDs in lab are made in comparison to databases:
  - IVD manufacturers are not required to include select agents or assess for select agents
  - Most databases DON'T contain select agents
  - Mis-identifications by commercial test systems are reported in literature
- If patient has compatible travel AND unexplained fever AND cultures are being sent → Notify lab of possibility